

Redistribution of Amyloid Deposits

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After 21 daily subcutaneous injections of 0.5 ml 10% casein, CBA/J mice were left untreated and evaluated periodically for 6 months for the development of amyloid in spleens, livers, and kidneys. At the end of the amyloid-inducing regimen, the mice developed moderate to heavy splenic amyloid, trace to light hepatic amyloid, and virtually no renal amyloid. Renal amyloid appeared about 2 months after cessation of the casein and then increased steadily, while splenic and hepatic amyloid gradually diminished. Six months after the cessation of casein, moderate amyloid deposits were observed in the kidneys whereas no, or only traces of, amyloid remained in spleens and livers. This renal amyloid was localized predominantly in the peritubular area and differed from the renal amyloid seen in rapidly induced disease, when it localizes dominantly in glomeruli. This phenomenon is interpreted in the light of possible redistribution of amyloid deposits from organ to organ, and the clinical and investigative significance of this possibility and others are discussed. (*Am J Pathol* 1980, 99:539-550)

AMYLOID DEPOSITS occur in a variety of organs, and once established usually stay in these sites over long periods of time without significant change.¹⁻⁴ Turnover of such deposits has been considered to be poor. Nevertheless, resorption of amyloid may indeed take place and has been reported clinically as well as under experimental conditions.¹⁻¹⁴ The possibility of redistribution of amyloid from organ to organ, however, has never been thoroughly evaluated. Recent biochemical and immunologic studies have determined that the major constituent proteins of amyloid are protein AL (homologous with the N-terminus of immunoglobulin that forms primary and myeloma-associated amyloid) and protein AA (a unique protein that constitutes a large part of secondary amyloid).^{2-4,15-20} Moreover, larger protein molecules related to those amyloid proteins, ie, lambda or kappa chains or larger moieties of the immunoglobulin (related to protein AL) and protein SAA (related to protein AA), are present in serum and may be precursors of AL and AA, respectively.^{4,21-26} In addi-

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tion, mixed reports concerning the presence of amyloid protein in the urine have been published.²⁷⁻³⁰ It therefore may not be unreasonable to speculate that there exists the possibility that amyloid may occur in the two different states—soluble and insoluble—and occur not only in tissue but in some form of precursor in serum and possibly even urine, though the latter is yet to be proven. Thus, the amyloid proteins within the body may have a degree of mobility.

During our long-term studies of murine amyloidosis models, which had originally been designed in order to analyze the pattern of resorption of amyloid, we have made observations that suggest that the redistribution of amyloid may well occur from organ to organ. The purpose of the present article is to describe this model and to call attention to the possibility of redistribution of amyloid and certain of its implications.

Materials and Methods

The mice utilized were of the CBA/J strain (Jackson Laboratory, Bar Harbor, Maine), female, 8–10 weeks old at the beginning of the experiment. Fifty mice were separated into groups of 10 per cage and fed on Purina Mouse Chow and given water *ad libitum*. Each mouse received daily subcutaneous injections of 0.5 ml 10% casein solution (Casein Hammersten, Nutritional Biochemical Corporation, Cleveland, Ohio) in a total of 21 injections or for 3 weeks^{31,32} and then were kept without any further treatment. A group of 6 mice were killed on the day after the last casein injection, and at 2 weeks and 1, 2, 3, 4, and 6 months after the cessation of the casein treatment.

The paraffin sections of spleens, livers, and kidneys were stained with Congo red and hematoxylin³³ and evaluated for amyloid deposition by light microscopy using conventional and polarized light.

We graded amyloid deposition as follows:

— or negative:	no amyloid deposits
trace:	minute amyloid deposits not exceeding 5% of the tissue area
1+ positive:	definite amyloid deposition replacing 6–25% the tissue area observed
2+ positive:	26–50% of the tissue area replaced by amyloid
3+ positive:	51–75% of the tissue area replaced by amyloid
4+ positive:	76% or more of the tissue area replaced by amyloid

In order to control for possible spontaneous occurrence of amyloid in this particular strain of mice, 30 mice were kept 10 per cage but received no treatment. Three mice were killed at times corresponding to those of the experimental group.

Results

The present results are summarized in Text-figure 1.

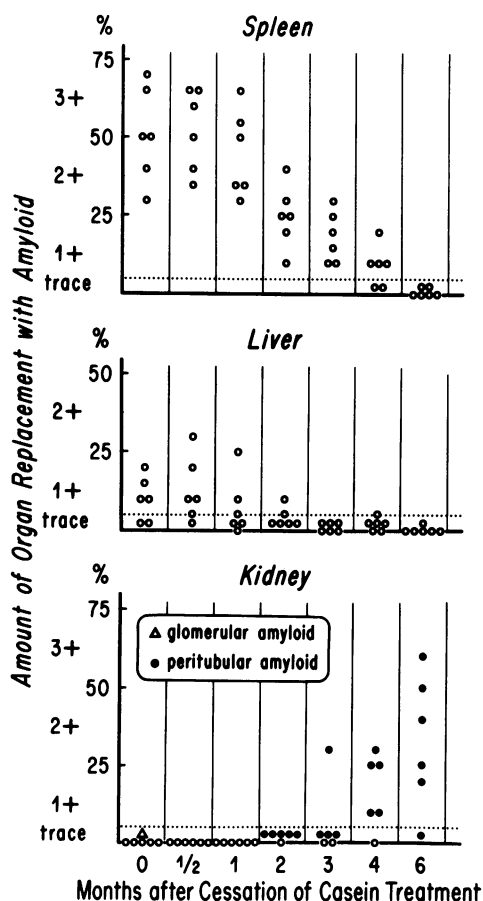
At the end of the casein treatment, the treated mice had 2⁺ to 3⁺ amyloid in the spleen (Figure 1) and trace to 1⁺ in the liver (Figure 2). In the

kidneys, however, only one out of 6 mice had a trace of amyloid in a few glomeruli (Figures 3 and 4).

At 2 weeks and 1 month after the cessation of the casein treatment, amyloid deposition in these organs showed no remarkable changes from that in those observed at the end of the casein treatment.

At 2 months after the withdrawal of the casein injections, significant changes were detectable. In spleens, homogeneous extracellular substance was observed in amounts comparable to those occupied with amyloid at the earlier stages of the present experiment. The major proportion of this extracellular substance, however, picked up Congo red only faintly. Under the polarized light, only scattered areas of the substance showed green birefringence, and amyloid deposition was judged to be 1⁺ to 2⁺. An increased number of cellular elements, mostly mononuclear cells, were

TEXT-FIGURE 1—Degree of amyloid deposition in spleens, livers, and kidneys of CBA/J mice observed at the specific times after cessation of casein (21 daily subcutaneous injections of 0.5 ml 10% casein solution).



present admixed in the substance (Figure 5). All the livers displayed amyloid deposits, but to a lesser degree than in the previous stages. In 5 out of 6 animals, the kidneys showed a trace of amyloid. The amyloid deposits in these kidneys however differed in that they were localized exclusively to the peritubular areas (Figure 6).

Three or 4 months after the casein treatment the trend of decreased splenic and hepatic amyloid and increased renal peritubular amyloid was even more striking.

Six months after the cessation of the casein only 2 of 6 spleens retained traces of amyloid, though all still showed considerably widened extracellular spaces, which were occupied with a homogeneous eosinophilic substance (Figure 7). Barely a trace of amyloid was found in 1 of 6 livers examined. In contrast, the renal peritubular areas displayed significantly increased amyloid, and the tubular epithelium often showed degenerative changes (Figure 8).

None of the mice of the control group showed amyloid deposits in any organ or at any time observed in the present experiment.

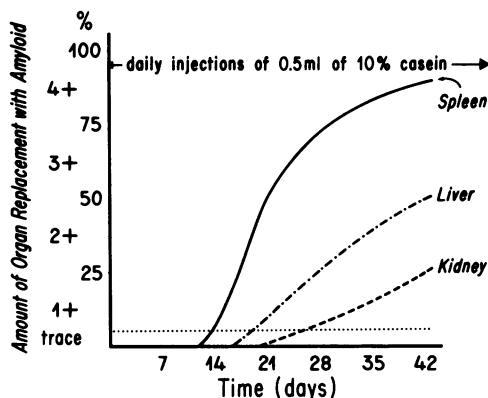
Discussion

Treatment with repeated injections of casein is the most popular method for the induction of amyloidosis in experimental animals.^{1-4,31} This model resembles human secondary amyloidosis in its light- and electron-microscopic appearance as well as its biochemical composition.^{1-4,16,19-22,31} In our laboratory, when CBA/J mice are treated with daily subcutaneous injections of 0.5 ml 10% casein solution, they usually develop splenic amyloid by 12-18 injections, hepatic amyloid by 16-22 injections, and renal amyloid by 20-26 injections. In the kidneys, the initial deposit of amyloid appears in glomeruli, and with several additional casein injections peritubular amyloid starts to appear in the inner medullar and papillary areas. With continued injections, amyloid deposition expands rather rapidly, and after 6 weeks on the regimen the animals begin to die (Text-figure 2).

In the present experiment, CBA/J mice first received 21 casein injections and then remained untreated. At the end of the casein treatment, the sampled animals had developed moderate to heavy splenic, trace to light hepatic, and virtually no renal amyloid. After the casein treatment was withdrawn, there was no remarkable change over the first month. In time, however, splenic and hepatic amyloid gradually diminished, while renal peritubular amyloid appeared and increased steadily (Text-figure 3).

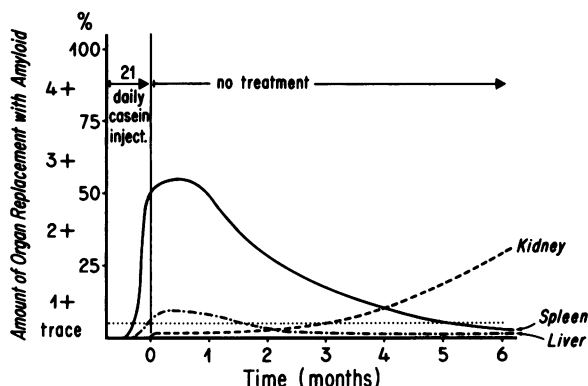
The gradual disappearance of splenic and hepatic amyloid after the cessation of the casein treatment has been attributed to its resorption.¹⁻¹⁴ Progressive accumulation of amyloid in the renal peritubular areas after

TEXT-FIGURE 2—Diagrammatic demonstration of development of amyloid in spleens, livers, and kidneys of CBA/J mice on an amyloid-inducing regimen (daily subcutaneous injections of 0.5 ml 10% casein solution).



the cessation of the casein treatment may be more complex. Possible explanations include: 1) that the amyloid is synthesized locally *de novo* or as the result of delayed effects of the casein treatment, and 2) that the amyloid is transported from elsewhere and simply trapped or reconstituted in the peritubular areas. Our data favor the second possibility for the following reasons. In the present study, the control group did not develop amyloidosis, and therefore the possibility of the spontaneous occurrence of amyloid in this strain of mice within the present period of observation is practically eliminated. There is now ample evidence suggesting that the serum amyloid protein A (SAA) has an important role in pathogenesis of secondary amyloidosis. Many investigators consider SAA a precursor or parent protein for the amyloid protein A (AA),^{4,21-26} although some suggest that SAA may be heterogeneous.³⁴ Elevation of SAA levels is always associated with active secondary amyloidosis or conditions predisposing to this disorder,^{4,23-26} although elevated SAA is nonspecific in many inflam-

TEXT-FIGURE 3—Diagrammatic demonstration of the evolution of amyloid deposits upon withdrawal of casein after 21 days of treatment.



matory conditions and does not necessarily lead to amyloidosis. In the murine amyloid model, the elevation of the SAA levels is found within a few hours after the first casein injection and reaches its peak within 12–24 hours. When casein injections are given daily, the level of SAA stays high. However, if the casein treatment is stopped, regardless of how many daily casein injections preceded, the SAA levels drop rather quickly and returns to normal within a matter of several days.²² The cellular immunologic aspects which undergo considerable change during the casein treatment also return to normal 1–2 weeks after withdrawal of the casein treatment.³⁵ These data suggest that the effect of casein treatment that evokes active amyloid production diminishes rather quickly after withdrawal of the treatment, and there are no specific data to suggest a long-lasting effect.

Review of the literature reveals several observations relevant to the present study. In the original study that introduced casein as an amyloid-inducing agent, Kuczynski reported resorption of splenic and hepatic amyloid after the cessation of casein treatment in mice.⁸ That amyloid in spleens and livers is resorbed after the amyloid-inducing regimen is withdrawn has been subsequently reported in mice and rabbits.^{1–4,6,7,10,11,13,14} Among these reports, moreover, all of which analyzed the kidneys, some scientists observed that after the cessation of the amyloid-inducing regimen, renal glomerular and peritubular amyloid 1) decreased, but at much slower rate than the splenic or hepatic counterpart; 2) stayed virtually unchanged; or 3) increased gradually.^{6,7,10,11,13} DeLellis et al⁶ studied long-term aspects of experimental murine amyloidosis induced with a single intraperitoneal injection of a Freund adjuvant containing added *Mycobacterium butyricum*. They observed that renal amyloid deposits (both glomerular and peritubular) progressively increased, while amyloid deposits in other organs decreased after initial deposition within the first few weeks. They even pointed out the possibility, among others, that fragments of amyloid deposits might be mobilized from other sites, transported to the kidney, and trapped within the glomeruli.

The present prospective study with controls more clearly delineates this probable redistribution and highlights it as a feature that may have importance as new modes of therapy are introduced. For example, if agents become available to solublize amyloid, will they be associated with increased risks of renal trapping of this molecule? Ultimately, the proven role of renal transplantation in the amyloid-overloaded kidney³⁶ may become more pertinent in part of a total therapeutic regimen. From an investigative point of view, one has to become more cautious not to confuse the primary sites of amyloid production with the secondary sites of amy-

loid deposition as the result of redistribution. Specific labeling of the amyloid molecule in the future may allow us to follow the resorption or redistribution of amyloid in a more precise fashion and to determine with certainty whether redistribution includes a component of *de novo* production as well.

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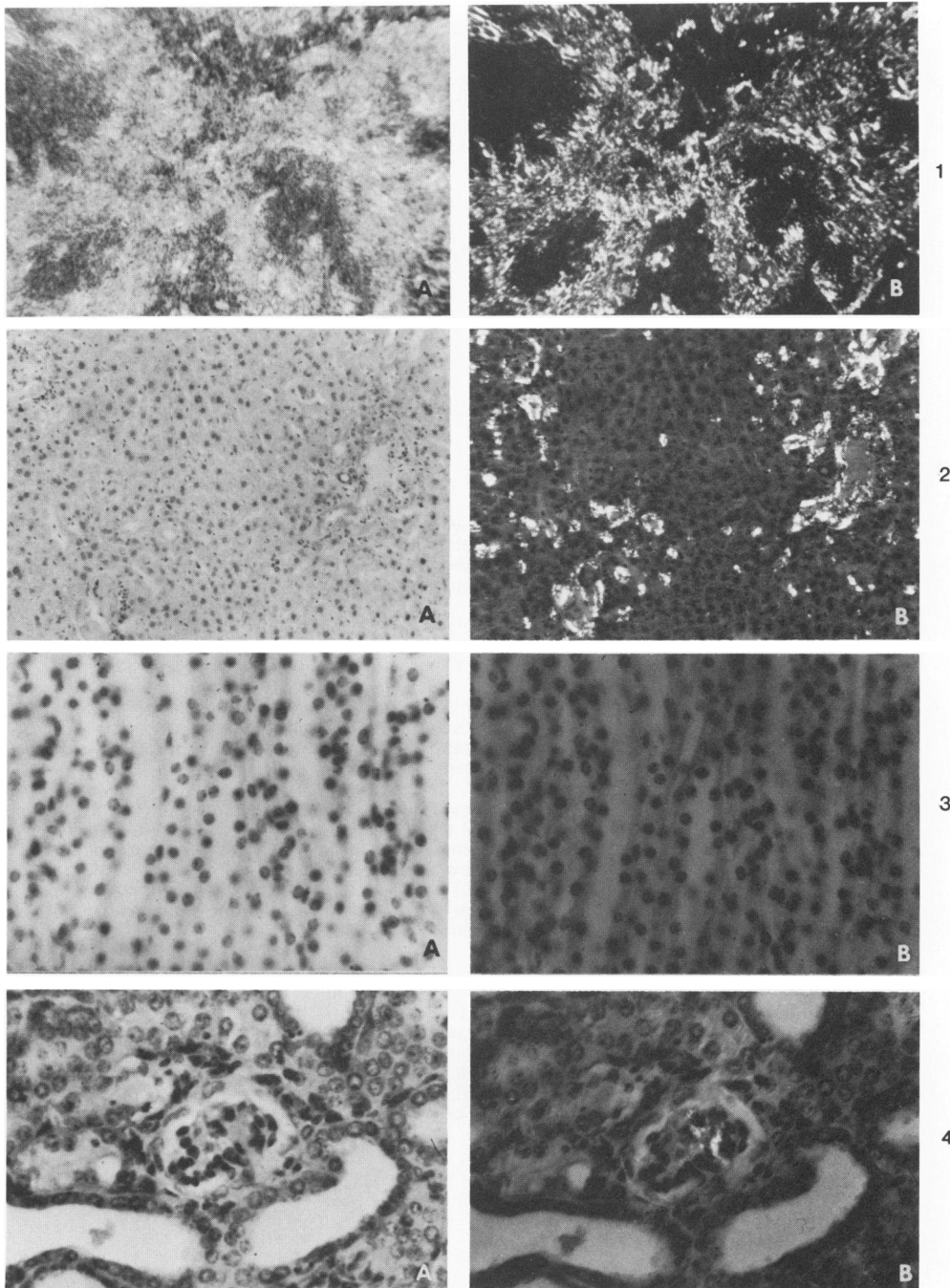


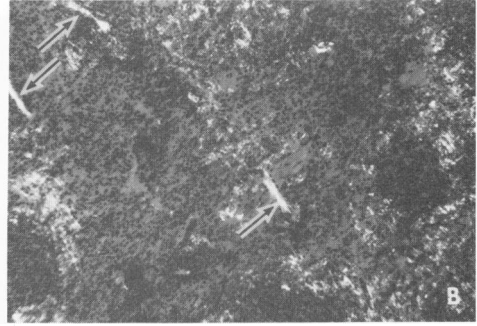
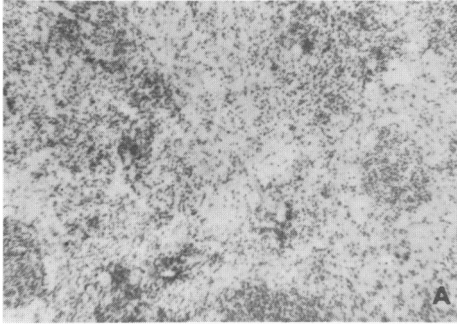
Figure 1A—Photomicrograph of spleen of a representative CBA/J mouse after receiving 21 daily casein injections. Congo-red-positive substance, amyloid, deposits heavily in the marginal zone, replaces large portions of the red pulp, and infiltrates into the white pulp as well. (Congo red and hematoxylin, $\times 70$) **B**—The same area as in figure 1A, photographed under polarized light. Congo-red-stained amyloid demonstrates bright green (when viewed in color) birefringence (white in black-and-white). ($\times 70$) **Figure 2A**—Liver of a CBA/J mouse that received 21 daily casein injections. (Congo red and hematoxylin, $\times 70$) **B**—Polarization photomicrograph of the same area as in A. Amyloid deposits around the blood vessels in the portal space and the central vein as well as in the space of Disse along the hepatic cell cord. ($\times 70$) **Figure 3A**—An inner medullary zone of kidney of a CBA/J mouse at the end of 21 daily casein injections, showing no sign of amyloid deposits or other significant structural changes. (Congo red and hematoxylin, $\times 270$) **B**—Polarization photomicrograph of the same area as in A, showing no birefringent deposits. ($\times 270$) **Figure 4A**—A cortical area of only the CBA/J mouse that had renal amyloid in a group of 6 mice that received 21 daily casein injections. A small amyloid deposit is suggested in the glomerulus, but not in other areas. (Congo red and hematoxylin, $\times 270$) **B**—The same area as in A, photographed through the polarizing microscope, showing a small glomerular amyloid deposit. ($\times 270$)

Figure 5A—Spleen from a CBA/J mouse that had originally received 21 daily casein injections 2 months after the cessation of the casein treatment. An amorphous substance occupies the areas comparable to those deposited with amyloid at the end of the casein treatment (as shown in Figures 1A and B). A large proportion of the substance is, however, only faintly Congo-red-positive. (Congo red and hematoxylin, $\times 70$) **B**—The same area as in **A**, seen with polarized light. Only scattered areas of the extracellular amorphous substance show classical green birefringence characteristic of amyloid. Note that the trabeculae (*arrows*) produce whitish (when viewed in color) birefringence and do not represent amyloid. ($\times 70$)

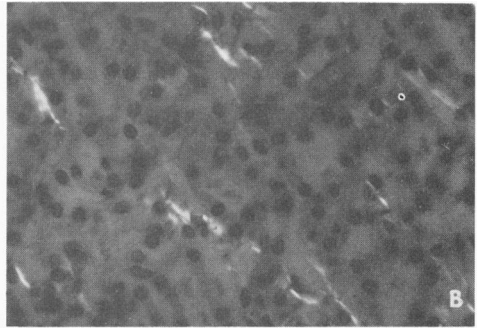
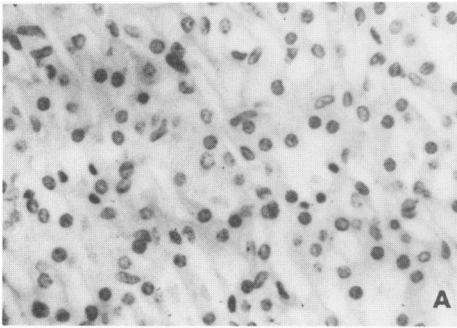
Figure 6A—Inner zone of the renal medulla from the same mouse whose spleen is shown in Figures 5A and B. Small amyloid deposits are seen in the peritubular areas. (Congo red and hematoxylin, $\times 270$) **B**—The same area seen in **A**. Under the polarized light, the peritubular amyloid deposits can be more easily identified. ($\times 270$)

Figure 7A—Spleen from a CBA/J mouse that had received 21 daily casein injections and then was left untreated for 6 months. The areas that were occupied with amyloid at the end of the casein treatment (see Figures 1A and B) still show a considerable extracellular amorphous material. However, the substance, except for a few scattered small areas, picks up Congo red only faintly. (Congo red and hematoxylin, $\times 70$) **B**—Through the polarizing microscope, the same area as in **A** demonstrates only a trace of green birefringent substance, amyloid. *Arrows* indicate trabeculae with whitish birefringence. ($\times 70$)

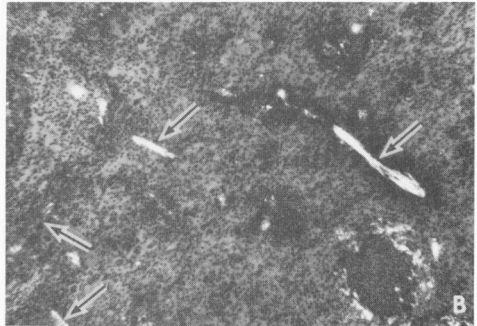
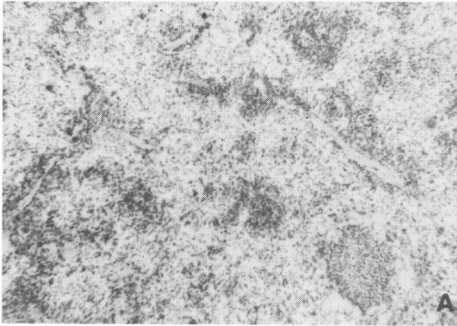
Figure 8A—The inner zone of the renal medulla of a mouse 6 months after the withdrawal of the casein treatment demonstrates considerable amyloid deposits in its peritubular areas. The tubular epithelia show degenerative changes. (Congo red and hematoxylin, $\times 270$) **B**—The same area as shown in **A**, observed under polarized light. Green-birefringent amyloid occupies a considerable portion of the peritubular interstitium. Note that the birefringence recorded in this micrograph does not represent the total amount of amyloid, since the micrograph was photographed at one axis of polarization. When observed at different axes, replacement of the interstitium by amyloid has been judged to be more than 60%. ($\times 270$)



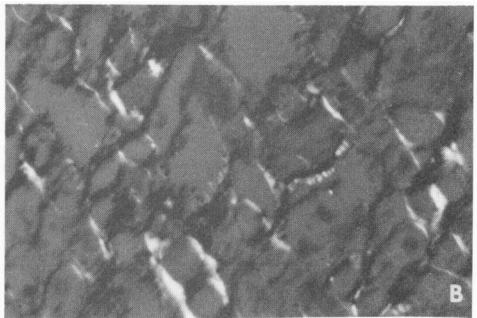
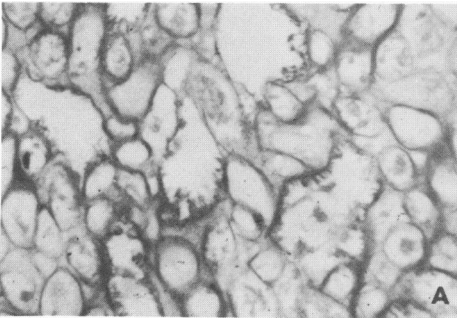
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